Environmental Chemistry and Microbiology

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Module - 9
Lecture - 48
Xenobiotics - II

Welcome everyone! This is lecture number 48 and this is the second part of Xenobiotics in module 9.

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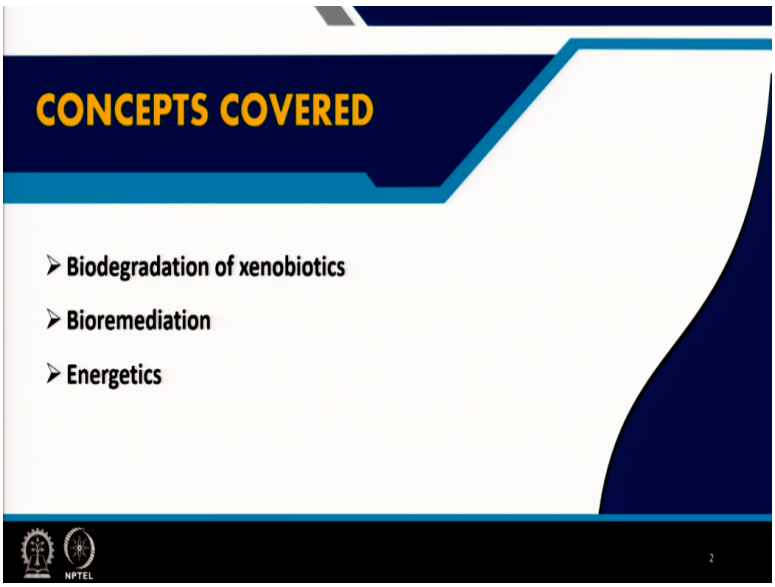

We are going to look at some of the remaining topics over here. The first thing we are going to look at is the biodegradation of xenobiotics. The second thing is how can we use this information about xenobiotics, about degradation, about the biochemical pathways; how can this knowledge be applied to bioremediate certain contaminated sites. And finally, I will introduce very briefly the topic of energetics. This is very important for understanding microbial diversity which is part of module 11 and 12.

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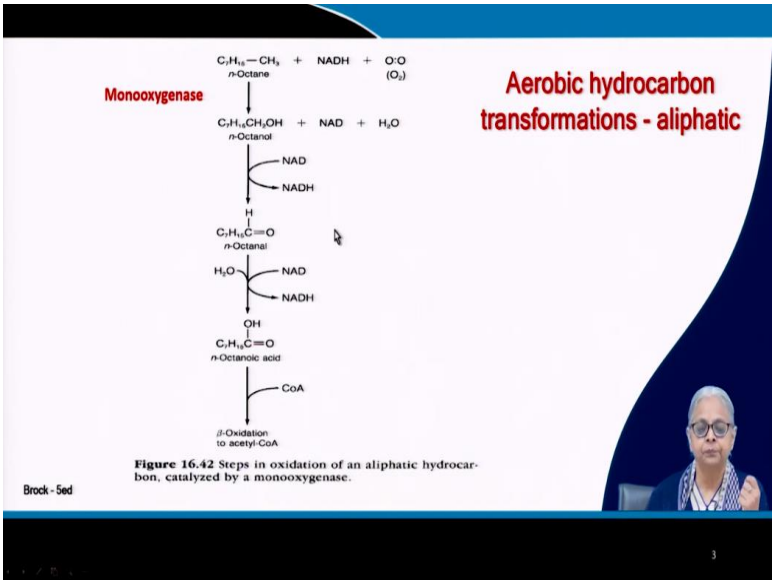

So, in the previous lecture, I stopped with the thumb rules that allow us to estimate whether a particular compound is going to be biodegraded or not. I already mentioned that aerobic hydrocarbons can be transformed. So, aliphatic aerobic hydrocarbons can be transformed under aerobic conditions, but not under anaerobic conditions. The reason for that is the very first step. So, this is the very first step which is considered the beta-oxidation of this aliphatic compound, and it is catalyzed by a particular enzyme called monooxygenase. Now, this monooxygenase and the beta-oxidation part is not possible under anaerobic conditions. So, if you have a saturated compound like n-Octane, it is not going to be possible to biodegrade it.

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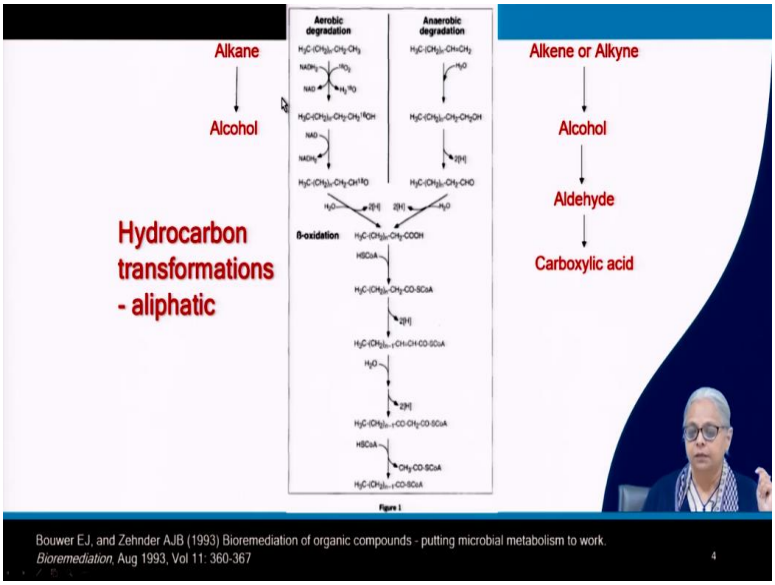

So, here we have a comparison between alkanes, alkenes, and alkynes. So, under aerobic versus anaerobic conditions, these alkanes will be converted to alcohols, and then they can enter; let me show you this. So, you have this octanol which is then further converted to

various other acids and these acids can enter the acetyl-CoA Krebs cycle. So, from there, they will be completely mineralized.

So, this is the first thing. So, you have aerobic biodegradation and beta-oxidation that can lead to complete mineralization of these alkanes, under aerobic conditions. Unsaturated compounds, aliphatics, alkene, and alkyne; so, double-bonded and triple bonded; they will be converted to alcohol, aldehyde, and a carboxylic acid, which can then enter the Krebs cycle and be completely mineralized. So, the unsaturated aliphatics can be converted under aerobic as well as anaerobic conditions. But they cannot be converted under anaerobic conditions, because that beta-oxidation step is not possible.

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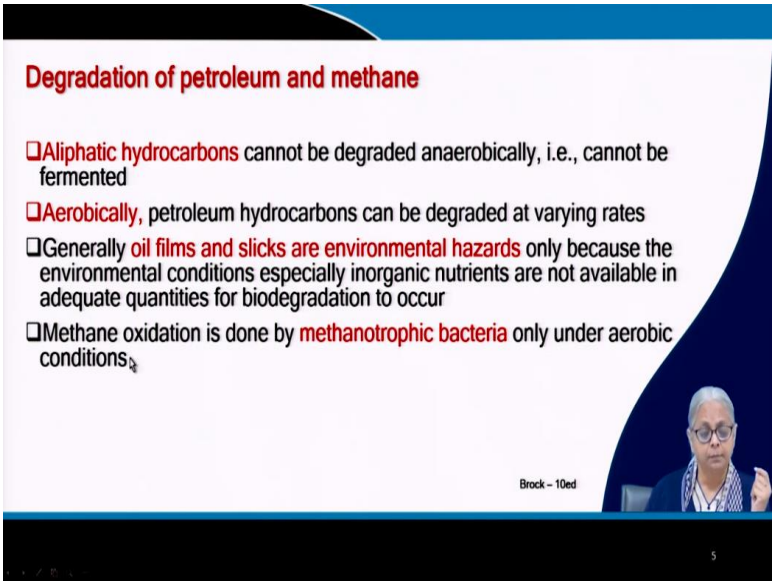

So, that is one part of the story in terms of the biodegradation of xenobiotics. Now, many of you have probably seen several media reports, very frequent, where you have petroleum spills, oil spills in the environment. So, high seas, there was just recently there was a ship, an oil tanker that collapsed and spilled millions of tonnes of oil into the sea. And there have been several other accidents in the past where shipping tankers carrying oil have spilled their content into the environment and caused enormous damage, mainly ecological damage.

Now, we might say that, okay, these bacteria are exposed to various conditions, can they degrade these oil spills? And the answer is yes, but it is not easy. So, let us take a look at what is required. So, degradation of petroleum and methane is possible. So, we have aliphatic hydrocarbons that cannot be degraded anaerobically. We have just seen that. Aerobically, petroleum hydrocarbons can be degraded at different rates.

Remember, the biggest problem with oil spills is that because of the lack of aqueous solubility, they form this layer on top. And when they form this layer on top, they cut off all the organisms that are living in the depth of the water are cut off from oxygen. So, this becomes a huge problem from an ecological point of view. So, oil films and slicks are environmental hazards only because environmental conditions, especially inorganic nutrients are not available and the other point which is not mentioned in the slide is the aqueous solubility; it forms a layer that cuts everything off. So, these are the 2 problems that make oil films and slicks an absolute environmental disaster. Methane oxidation is possible. So, if you have an area where methane is being generated and you want to find a solution to it, methane oxidation by methanotrophic bacteria under aerobic conditions is possible.

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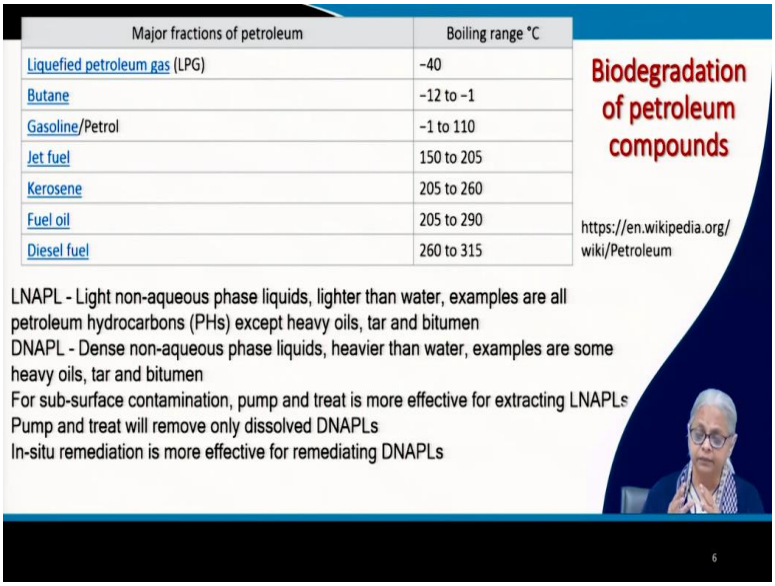

Now, we are all familiar with news reports where petroleum or petroleum-related compounds and oil spills have happened, and they have contaminated either the marine environment, the terrestrial environment, or even the subsurface environment. Now, what are these compounds that we are dealing with? So, these petroleum compounds, most of you have probably learned at some point that petroleum is not just 1 compound, it has thousands of compounds in it, and they range in size as well as in terms of their physical and chemical characteristics.

There is a very wide range of compounds that are present in petroleum. So, if we take the lightest part of petroleum that includes liquefied petroleum gas, it has a boiling point that is lower than 0. And you know, your LPG is basically propane and butane, and that has a boiling point range of -12 to -1°C. The petrol that we use in our vehicles has a boiling point range from -1 to 110°C.

Jet fuel is from 150 to 205°C. Kerosene, fuel oil, all these have higher boiling points 205 to 260 or 290°C. And finally, we have diesel fuel which is considered heavy, but not the heaviest. So, you have 260 to 315 (deg C). Now, these are in terms of the boiling points. These are the petroleum compounds that we see around us and we see, these are the ones that are visible to us.

You also probably know that in terms of specific gravity, you have certain compounds within the petroleum mixture, you have certain compounds that are lighter than water and these include all of these compounds that are shown in this table over here, they are all lighter than water, which means that when they are spilled into a marine or an aquatic, any aquatic environment, in fact, subsurface, marine environment, wherever it is, they will float to the top. So, all petroleum hydrocarbons except the heavy oils, tar, and bitumen, their specific gravity is less and they are all lighter than water. That is, all these compounds are called light non-aqueous phase liquids. Then we have dense non-aqueous phase liquids which are defined as compounds within the petroleum, which have specific gravities greater than that of water.

So, they are heavier than water and they will sink to the bottom in any aquatic environment. So, examples include heavy oils, tar, and bitumen; and none of them is shown over here. A very large number of carbon atoms are present in these dense aqueous phase liquids. They are called DNAPLs and LNAPLs for the lighter ones. And all the lighter ones have carbon numbers less than I think 50 to 70, and DNAPLs have C 70 or higher number of carbon atoms.

That again goes back to those leaking underground storage tanks which contain petroleum compounds. So, let us say, a tank ends up spilling its contents into the subsurface; what will happen is; for some of you who may be knowing this, petroleum is not a single compound, it is got hundreds and thousands of compounds in it. It has got a number of compounds. And these compounds, all have different specific gravities, their density is different, their solubility is different, all kinds of things are; their basic chemical characteristics are very different. And what will end up happening in the subsurface is that you will get light non-aqueous phase liquids. So, LNAPL stands for light non-aqueous phase liquids and DNAPL stands for dense non-aqueous phase liquids. So, this light LNAPL will obviously swim at the top, it will float, and the heavier materials; you are all familiar with bitumen and tar and all these; these are heavy residues of petroleum compounds. So, you can see that. And if you take petrol, fill it in a jerry can or a bottle, you can probably see some of that very clearly over a period of time. Regardless, in water, their behavior is going to be very different.

So, you will have the light non-aqueous phase liquids. Remember they are all non-aqueous phase liquids; they do not like to be in the water. So, even though this is a saturated groundwater situation, you get these light non-aqueous phase liquids which will be at the top and the dense liquids will find a way to the bottom. Now, how do you remediate this kind of situation? Once the accident has happened or this area has been contaminated, what can you do?

One solution is pump and treats for extracting the compounds that are at the top. That is one. The second is, you can try to extract the DNAPLs as well. The dense non-aqueous phase liquids can be; one can try to extract them, but only the ones that are in the dissolved phase will be extractable. The remaining part will remain as a pool of contaminant at the bottom, and that is very difficult to remediate. So, the only possible strategy may be in situ remediation for remediating DNAPLs. LNAPLs are much easier.

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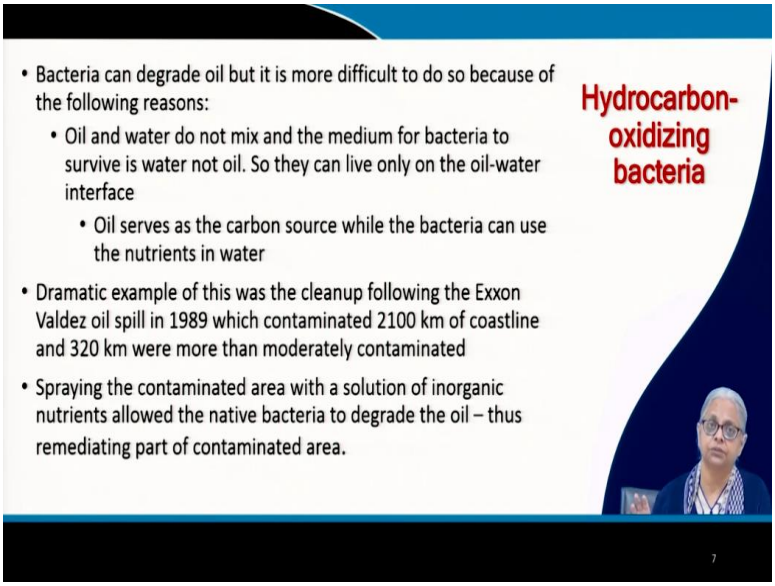

So, we now come to another issue and that is the ability of bacteria to degrade the oil. Now, oil and water do not mix, and because of that, it is very difficult to degrade the oil. The example that I am going to talk about here is the Exxon Valdez oil spill, which happened in 1989. And I would like to point out that there are several photos on the internet as well as in almost every textbook which shows the contaminated beach areas, the entire coastline that was contaminated by the oil. There are pictures of birds and other animals that were covered with oil. So, all these kinds of pictures provide a very dramatic example of what happens when you have an ecological disaster of this proportion. This particular slide; and goes back to the Exxon Valdez spill in 1989. Some of you might remember or may have seen media reports and so on, where this huge oil tanker basically spilled most of its contents along the

entire coastline of Alaska. And you can see the state of the beaches, it is completely covered with oil. And the birds, the fish, all of them are affected by this. So, it is like a major, one of the biggest ecological disasters that we have known in the last 50 to 70 years. And what I want to show you, I want to point out here that; you know, we normally think of oil as being difficult to biodegrade, but here is proof that it can be done simply by providing inorganic nutrients.

So, you have this entire area looks black (see either textbook for photos of this oil spill). It is black because of the oil layer. So, here you have all this black area. And you have this light area. This light rectangle which is shown by this arrow is light. And this area was sprayed with a mixture of inorganic nutrients. So, simply adding nutrients was sufficient to encourage the native microbial community in that area to start utilizing the oil.

So, this is how this part at least of the contaminated area was remedied. That is one thing. And the second thing is just a demonstration of the fact that because you have oil in water, so it will form droplets. And these droplets are going to be very difficult to biodegrade. So, here you see the bacteria are all at the interface. The oil-water interface is where all the bacteria are. And they can degrade it, but it takes longer because of solubility issues.

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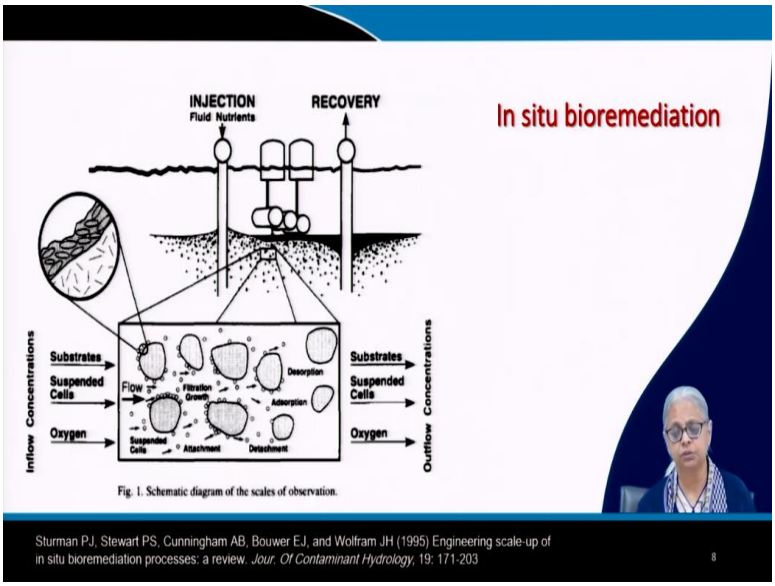

A little bit more about bioremediation. So, what can we do for remediating contaminated subsurface environments? So, what is required is often just nutrients. And in some cases, by adding acclimated microbial consortia, you can remediate the compounds that are in the subsurface, that have contaminated the subsurface. So, you have substrates, you have suspended cells, you have oxygen, all of this can be added to the subsurface along with inorganic nutrients, allow and encourage the native microbial community to grow.

And if the compounds are resistant or not degradable by the native community, you may have to add acclimated microbial consortia to it. So, this is one possibility. And then you have to recover the contaminated material and either utilize it or treat it further.

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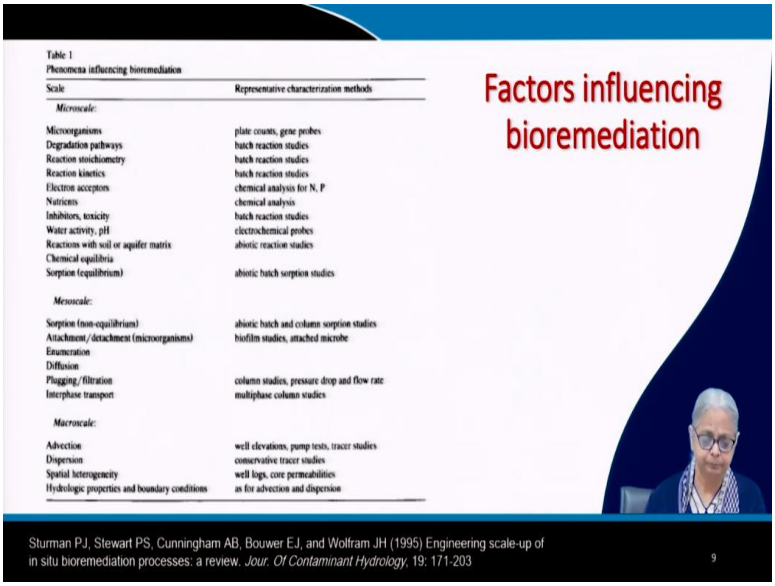

There is a number of factors that influence the effectiveness of bioremediation. So, you have microscale, mesoscale, and macroscales factors. So, the nature of the organisms, the degradation pathways, the reaction stoichiometry, kinetics, electron acceptors, nutrients, inhibitors, water activity, pH, temperature, reactions with the soil and aquifer matrix, chemical equilibria, sorption; are the micro-scale factors that will determine what happens to the microbes and the compound at the micro-level.

We have done studies; there are lots of people; there is a huge amount of literature out there that shows that all of this is possible, not just in the lab, but also in the field. And I will show you some more proof of that. Mesoscale: Sorption, non-equilibrium as well as equilibrium; attachment and detachment of microorganisms; how do you quantify microorganisms? what is the diffusion rate of the compound? Are the microorganisms going to be filtered out or are they going to cause clogging of the subsurface pores? what is interfacial transport? so, whether the compound is going to remain in water; will it absorb the rock material or the aquifer material; all these things need to be understood. And then at the macroscale, you have advection, dispersion, spatial heterogeneity, hydrologic properties, boundary conditions; all these things are required.

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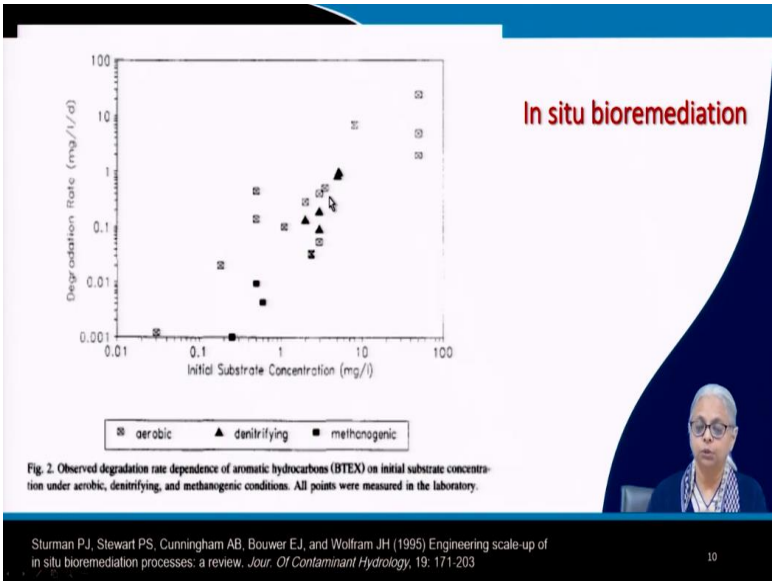

Here is another graphic from the same paper and we have the degradation rates of four aromatic hydrocarbons: benzene, toluene, ethylbenzene, and xylene (BTEX). So, BTEX; these are common in petrol; and under aerobic conditions, denitrifying conditions, and methanogenic conditions. So, you have the initial substrate concentration and the degradation rate. So, this is what you see. you can see that under aerobic conditions, the microbes can degrade these compounds, over a wide range of substrate concentrations. Under denitrifying conditions and methanogenic conditions, there is a smaller window, but it remains possible.

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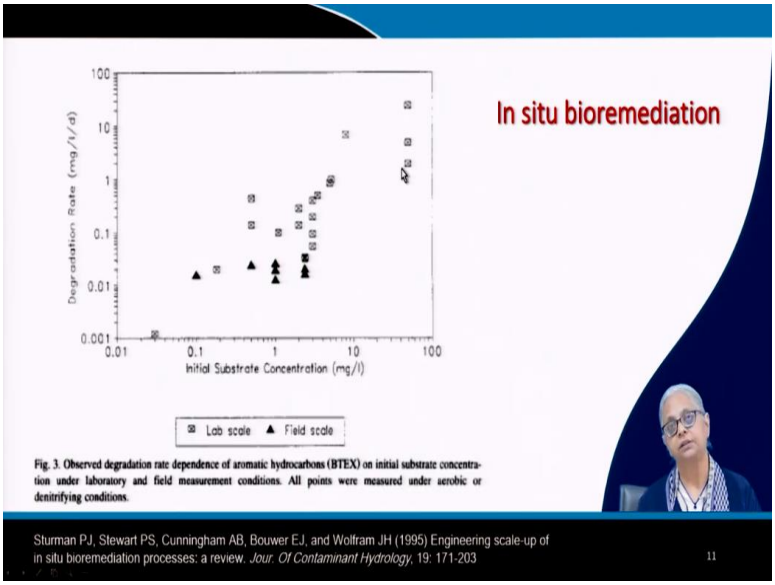

Then we come to lab-scale versus field scale. I just said that these studies have been done for a very long period of time by many different researchers in many different countries and these are possibilities. So, we can do lab-scale studies. You can see, as the concentration of

the substrate increases, the lab-scale study shows that the degradation rate in general increases with an increase in substrate concentration.

What happens in the field is a different matter. In the field, you will find that there is more or less very little change in the degradation rate of these compounds in the field. Now, the field is never going to be half as efficient as the lab. In the lab, we have the ability to control all of these factors. So, all the factors that are mentioned here, we generally do lab studies, and we control all of them. So, under those controlled conditions, you are going to get a very high efficiency of degradation and so on. But in the field, you have no control, you have absolutely no control over all these factors. The field factors are going to control the nature of degradation and the efficiency of degradation. So, you find that there is very little variation, no matter what the substrate concentration is. And these were done under aerobic as well as denitrifying conditions. So, the bottom line here, it is definitely possible to have good in situ bioremediation. But will the field results replicate results in the lab? That remains to be seen. That cannot be predicted I think a priori.

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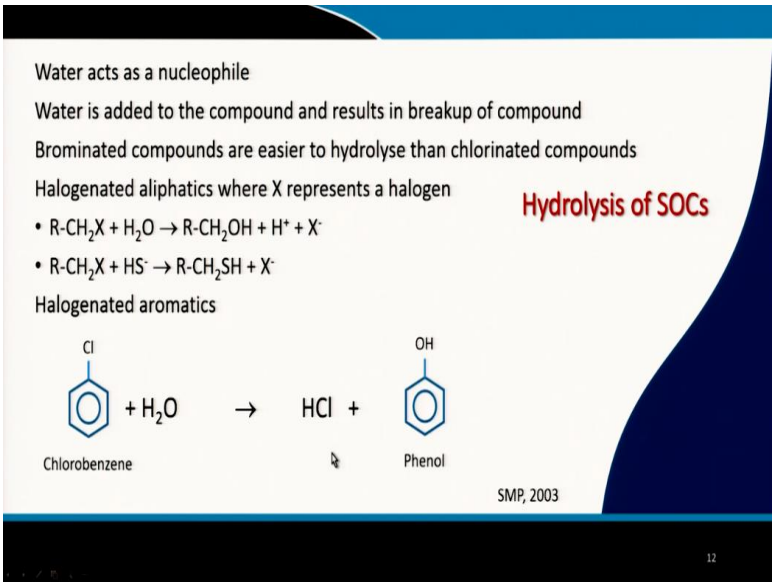

So, we have different types of reactions. Coming back to the chapter that I started with, chapter 6 on Biochemistry from Sawyer, McCarty, and Parkin. So, we will come back to that and look at some of the reactions that are possible, that allow the degradation of these compounds. Some of you may be familiar with the idea that chlorinated compounds are generally considered more resistant to biodegradation compared to non-chlorinated compounds.

So, here we have halogenated aliphatics as well as aromatics. So, when water or sulfide is present in the environment, it will substitute for the halogen and cause dehalogenation of

these chlorinated compounds. That is essential for further biodegradation because these chlorinated compounds are toxic to the bacteria. So, the first step is hydrolysis where water works as the nucleophile. It is added to the compound and results in the breakup of the compound, and makes it more biodegradable. There are several other examples with esters, with amides, carbamates, and phosphoric esters. So, these are some pesticides, furan, parathion; these are all examples.

So, we have another example over here besides the aliphatics. So, we have halogenated aromatics like chlorobenzene. So, when chlorobenzene is present along with water, the water again acts as a nucleophile, and you get dehalogenation of chlorobenzene, you get the formation of phenol and hydrochloric acid. Now, phenol is I think, a little easier to biodegrade compared to chlorobenzene.

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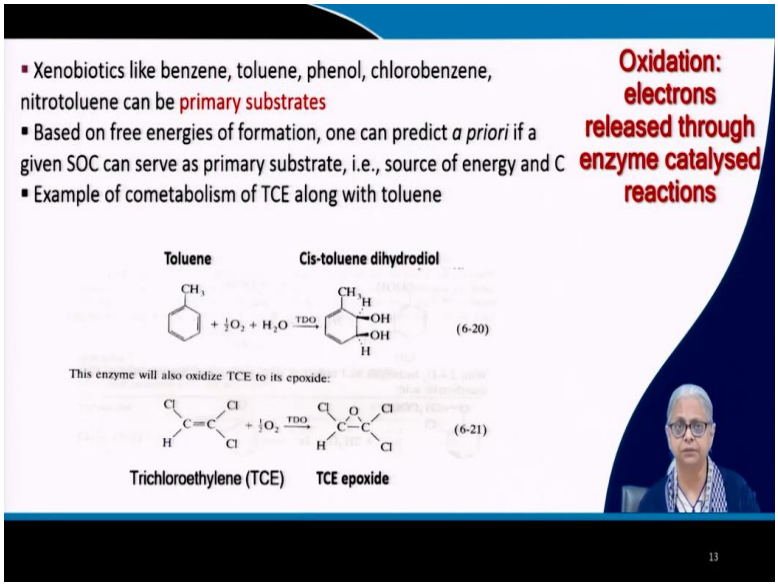

I have already explained this particular set of reactions where you have a primary substrate and a second substrate. So, you get cometabolism with the combination.

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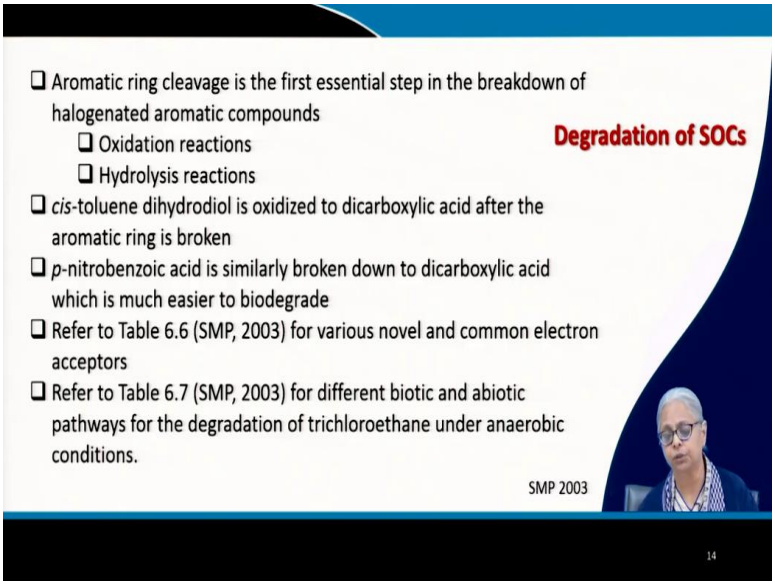

Here you have another example of aromatic ring cleavage. So, you know that the aromatic ring is the most stable chemical structure. To break it, to make an aromatic compound biodegrade, requires breaking of that aromatic ring and that is the aromatic ring cleavage. So, oxidation reactions, hydrolysis reactions have to happen. So, here you have water acting as; it allows the breaking of the aromatic ring. You get 2 carboxyl functional groups.

And then you have other examples over here. Now, once the ring is broken, then it becomes very easy to degrade the compound further. So, that is the first step; the most crucial step. And then you have para nitrobenzoic acid which can be oxidized to dicarboxylic acid. So, para nitrobenzoic acid can be oxidized to dicarboxylic acid. So, you have this para benzoic acid. The nitro group is removed first. And then it is further degraded and the ring is broken.

And these are all water is one of the reactants. So, you have 2,4-D, hydrolysis, reductive dealkylation, and all these reactions. And like I said, organic chemistry is not my forte, and I am not going to do this. So, this is for people who are interested in organic chemistry and they know how easy it is for them. This is like I said, it is not my area of expertise but these possibilities exist. And I just wanted to point out that these are the possibilities that do exist and allow the biodegradation of aromatic compounds, saturated and unsaturated aliphatics, and so on.

This is another table of half-reactions and reduction potentials with novel and other electron acceptors. So, as I said, people have been doing these studies; they have been studying various chemical compounds that have contaminated the environment and what are the possibilities. You can have a combination of abiotic and biotic reactions for remedying contaminated areas. And these are all lab studies, they are done with single compounds and often single species of bacteria. But in the field, you are dealing with multiple sets of

compounds and multiple bacteria. So, life in the field is very difficult. We all know that. But one has to start with either lab studies and then take it to the field and so on.

So, regardless of what can be done, I just want to point out again that all these possibilities exist in microbially as well as chemical reactions. And it takes a fair amount of work to understand whether a particular chemical compound can be biodegraded more easily or it can be treated chemically more easily. So, these are some of the challenges that I wanted to point out and that some of you can get into.

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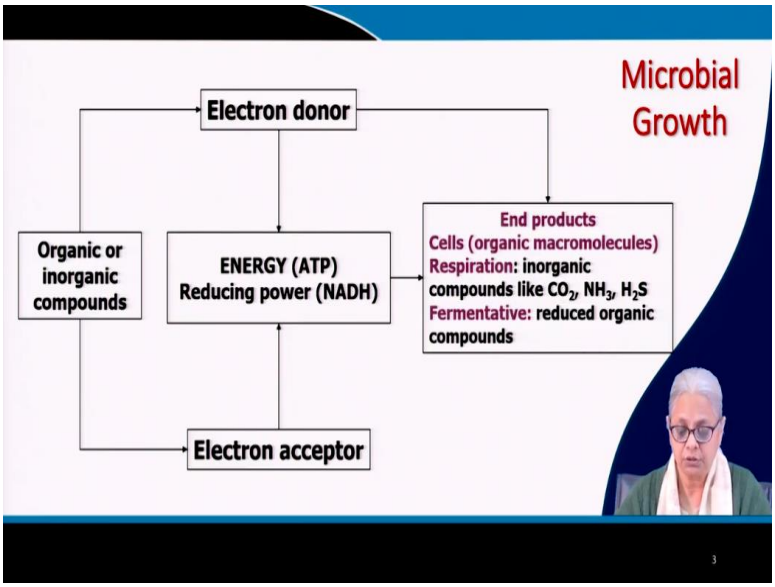

Now, that we have seen the biodegradation of synthetic organic compounds, we will take a look at another very important aspect of microbial metabolism. With all this information in the background, we now want to be able to quantify the end products of microbial growth. So, in the next few slides, we are going to look at that. So, when we think about microbial growth, we have already seen that the first thing that is required is the coupling of electron donors with electron acceptors. And this coupling is going to produce 2 things. One is ATP. And we know that ATP is a high-energy compound, and that is how the cell is going to store its energy. And the other thing that is generated is NADH. NADH is the reducing potential or the reducing power. So, that is the driving force for the electron transport chain. So, these 2 things are what are going to be generated when you get the coupling of the electron donor and the acceptor.

Now, the electron donor can be an organic compound or it can be an inorganic compound. And in the subsequent part of this lecture, we are going to cover exactly all these combinations. So, what are the end products of the coupling of electron donors with acceptors. The first major end product is new biomass. So, we know that the biological

requirement for reproduction results in a certain amount of new biomass. So, this new biomass is quantifiable. In the lab, when we do experiments with different electron donors and acceptors, we are generally in a state to quantify the new cells or new biomass that is created. And these new cells or new biomass are nothing but organic macromolecules within the cell.

Now, if the biochemical pathway is respiration, whether it is aerobic or anaerobic, what are the end products of those reactions? The end products are most likely to be carbon dioxide, ammonia, and hydrogen sulfide. If they are coming through a fermentation reaction, then we are not going to get these gases. Maybe under certain conditions, you will get CO_2 and methane; or you will get reduced organic compounds, for example, the alcohols or lactic acid and so many other acids which I have already shown in the previous topic. So, you get certain amounts of reduced organic compounds, along with some of these gases.

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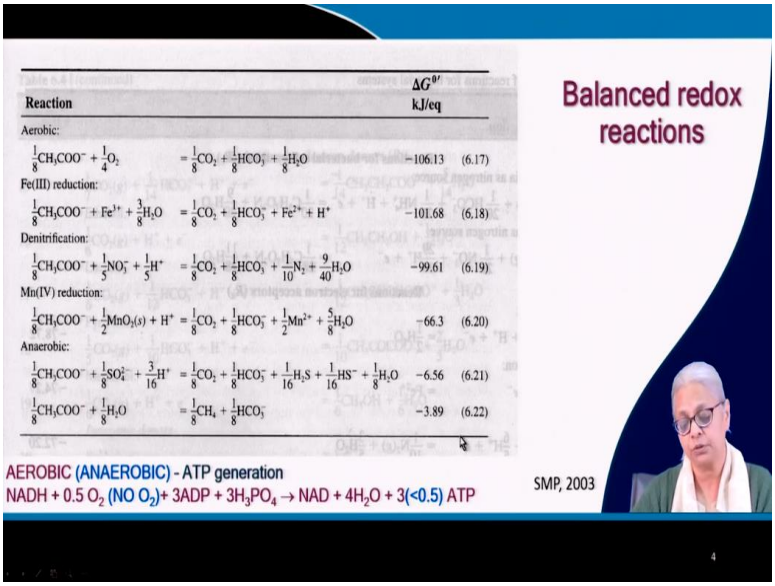

So, what you see over here are redox reactions where the starting compound is not glucose. We have been looking at glucose up to this point and it is a C6 compound. Here we are taking another starting compound that is much simpler than glucose, and this is acetate. So, CH_3COOH is our starting compound or the electron donor. And the same principle that we saw in the electron tower where you combine glucose as the electron donor with oxygen as the terminal electron acceptor; when you combine them, you get the highest energy yield. And in that case, it was E0 dash values. Here we have ΔG_0' values. The units are kilojoules per equivalent because these reactions have been written. These are half-reactions that have been combined, and they are written in terms of the number of electrons transferred, and then divided by the number of moles of electrons. So, this is electron equivalent. So, if you have 8

electrons being transferred from acetate to oxygen, then you divide the entire thing by 8 to give you kilojoules per electron equivalent.

So, this is our first reaction, and that is the one that has the highest ΔG or highest negative ΔG value, ΔG_0' value. The next terminal electron acceptor is Fe^{3+}. So, Fe^{3+} can be reduced to Fe^{2+}. You can write a balanced reaction for that and you can find out. So, here you will get ΔG_0' of -101.68.

If your terminal electron acceptor is a nitrate, then you get nitrogen gas at the end. And this is our denitrification reaction, and your ΔG_0' value is -99.61 kilojoules per electron equivalent.

Then we come to manganese(IV). This is manganic iron. So, manganic is converted to manganese, and the ΔG_0 value here is -66.3.

These are all negative values, which means there is sufficient energy released in these reactions for ATP to be generated and for the bacteria to be able to utilize this energy for maintenance, growth, maintenance, and reproduction.

Then we come to anaerobic reactions. So, under strictly anaerobic conditions, if sulfate is there in the environment, it will be converted to hydrogen sulfide. The energy yield is very small; it is only -6.56, but it is sufficient to allow the bacteria to survive.

And finally, we come to another situation where there is no oxygen. The acetate will ferment, will be fermented by the bacteria resulting in methane and CO_2, and the energy yield is the lowest.

We know that in terms of moles of ATP for glucose, it was 2 moles of ATP per mole of glucose. So, you can see in terms of ΔG for acetate as well, this is the lowest energy yield.

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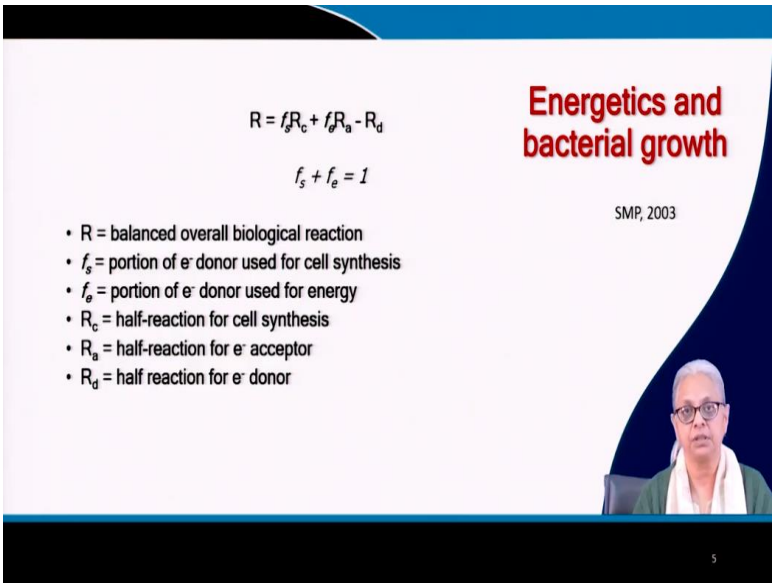

How do we quantify all these things? So, we know a lot based on the thermodynamics of these reactions, and we can utilize this information to determine the overall reaction and to determine the amount of end product. So, if I want to determine the gas produced, especially biogas, so I want to know how much methane is produced. If I want to know how much biomass; because when you do it at a full-scale level, you want to know how much sludge is going to be produced.

So, that sludge is nothing but biomass. And that biomass needs to be quantified. So, you can use this method for quantifying or estimating what is the maximum possible gas production; what is the maximum possible sludge production; what are the other end products; any additional chemicals that need to be added to the process. So, all that can be quantified based on this method. So, here is our overall reaction.

This is the balanced overall biological reaction. Now, this balanced reaction has 3 components to it. R_c is the half-reaction for cell synthesis. R_c - c stands for cell synthesis. And what we need to remember is that the electron donor is partly going to be converted to biomass and part of that electron donor in combination with the electron acceptor is going to be converted to energy. So, all of the compounds is not going to go for energy, some of it is going for cell synthesis. So, this is the half-reaction for cell synthesis. The second one is the half-reaction for the electron acceptor. That is where the combination happens of the electron donor and electron acceptor. Now, f_s and f_e. So, which part or how much, what fraction of the electron donor is going for cell synthesis, and what fraction goes for energy? That is, the two together are equal to 1, and you have to multiply this. So, $f_sR_c + f_eR_a$ is the first part. Then we come to R_d which is the half-reaction for the electron donor. Now, when we add up the 2 reactions, electron acceptors and electron donors, this has to be reversed. You will see why that has to be reversed, and it has to be written in negative form. It is simply to balance the electron donor and the electron acceptor because all the half-reactions are written in terms of the release of electrons. So, you have to reverse the reaction to putting it in terms of donation and acceptant.

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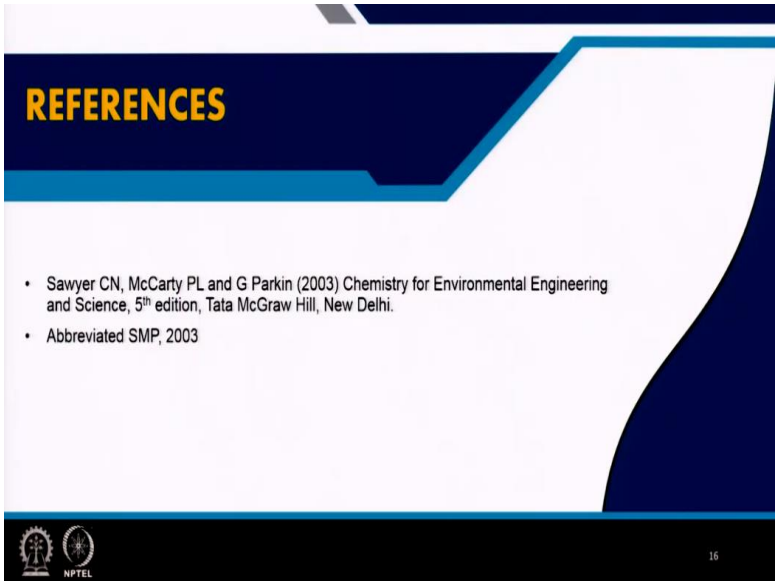

So, I will stop at this point. All this material comes from the same book chapter and the papers that are referenced. Thank you for your attention. I will stop here.